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BIODEGRADATION OF JET FUEL BY AQUATIC MICROBIAL COMMUNITIES

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16. ABSTRACT

This paper describes laboratory experiments that studied the fate of jet fuel in several types of situations that could be encountered in the field. Benzene, toluene, and p-xylene were the only components of the fuel that dissolved in the water to significant concentrations. All three compounds volatilized within 24 h and, thus, did not remain in the water long enough for microbial degradation to affect their fate. Inclusion of sediment (500 mg/l dry weight) did not retard the disappearance of the fuel components, and rates of disappearance were identical in controls sterilized with $HgCl_2$.

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INTRODUCTION

Biodegradation of petroleum in situ is an important factor that should be considered in the development of microbially-enhanced oil recovery programs. Many of the lighter aliphatic and aromatic components of petroleum are biodegradeable under appropriate conditions (1). Such biodegradation, if extensive, can reduce the value of the oil. In other instances, however, biodegradation of alkanes and concomitant production of emulsifiers can reduce the viscosity and increase the mobility of the oil thereby facilitating its recovery (9).

Biodegradation must be studied in the laboratory under controlled conditions so that its effects can be distinguished from those of abiotic factors. We know which of the lighter hydrocarbons are biodegradeable (1) but little quantitative data are available concerning the biodegradation rates of individual components of petroleum in mixed cultures. Gasoline and aviation fuels are composed of the most volatile and biodegradeable components of petroleum. Because of their potential for accidental release into surface and groundwater we have studied the fate of such fuels in aquatic systems. We chose JP-4 as the primary test fuel because of its extensive use in both military and civilian aircraft and because its composition is well-known (6).

Several processes can be expected to affect the fate of JP-4 in the aquatic environment. The heavier components tend to sorb to organic particulate matter and sediment, whereas the lighter components are more susceptible to volatilization. We have designed our laboratory test systems to allow us to assess the relative influences of biodegradation, sorption, and volatilization on the fate of the fuels.

MATERIALS AND METHODS

Sampling Sites. Water and sediment were collected at sampling sites in the Escambia River, Bayou Chico, and Range Point salt marsh near Pensacola, Florida. Salinity at Bayou Chico and Range Point ranged from 10 to 20 parts per thousand. Water was collected, filtered through a 3.0 µm membrane filter (Nuclepore Co. Pleasanton, CA), and stirred overnight at room temperature. The top 3 to 5 cm of sediment and associated detritus were collected at each site along with the overlying water. The suspension was passed through a 2 mm screen and particles of sand were allowed to settle out. The resultant organic sediment slurry was decanted and then stirred overnight at room temperature.

Fate Test. Fuels were incubated under four sets of conditions during quiescent fate tests: (1) active water (AW) contained filtered water from the sampling site; (2) sterile water (SW) contained filtered water sterilized with 0.1 percent $HgCl_2$; (3) active sediment (AS) contained sediment and filtered water; (4) sterile sediment (SS) contained sediment, 0.1 percent $HgCl_2$, and filtered water from the test site. Tests were conducted in duplicate 150-ml milk dilution bottles. The final volume of liquid in each bottle was 25 ml. JP-4 (250 µl) was added to the surface of the water in each bottle, and the bottles were capped and shaken in a horizontal position for 10 minutes at 150 rpm. The caps were then removed and the bottles incubated horizontally and undisturbed. Two sets of control bottles received no fuel and were used to monitor microbial populations and background contamination of environmental samples. After incubation for appropriate intervals, the contents of each bottle were extracted with 10 ml of CS_2 that contained hexadecane (773 ng/ µl) as an internal standard. The extracts were placed in glass vials, sealed with silicone septa, and stored at -20°C until analyzed.

Chemical Analysis. Fuels were analyzed by high resolution capillary column gas chromatography with a Hewlett Packard 5730 gas chromatograph equipped with a flame ionization detector and cryogenic unit. The capillary column was 25 meters long, 0.2 mm i.d., fused silica with methyl silicone bonded phase (Scientific Glass Engineering BP-1). Hydrogen was used as the carrier gas. Inlet and detector temperatures were 250°C. The temperature program for JP-4 began at 30°C with an 8 minute hold followed by an 8°C/min increase to 220°C. Fifteen representative components of the fuel were selected for quantitation (Table 1). Data integration and storage were performed with an HP3357 computer.

Microbiology. Heterotrophic bacteria were enumerated by a standard five-tube most-probable-number (MPN) technique (2). The enumeration medium contained 1 g of yeast extract and 5 g of Bacto-Peptone (DIFCO) per liter. The pH was adjusted to 7.6 prior to autoclaving. Salinity values were adjusted by the addition of an aged sea salts solution. MPN tubes were inoculated with 1.0 ml of an appropriately-diluted sample. Tubes were incubated at room temperature and examined for turbidity after 2 weeks. Hydrocarbonoclastic bacteria were enumerated by the ^{14}C-MPN technique described by Lehmicke et al. (3) with [^{14}C]-hexadecane as the carbon source.

Materials. JP-4 was obtained from Maj. D.D. Potter, Wright Patterson AFB, Ohio. Standards for the compounds listed in Table 1 were obtained from Aldrich Chemical Co. n-[1-^{14}C]-Hexadecane (specific activity 235 μCi/mg) was obtained from Amersham.

RESULTS AND DISCUSSION

Simple laboratory experiments were designed to study the fate of the fuel in several types of situations that could be encountered in the field. Shake flask studies provided a simple system for the study of the fate of fuels released in open water. We prepared water-soluble fractions of JP-4 and incubated them in gently-shaken flasks (75 rpm). Benzene, toluene, and p-xylene were the only components of the fuel that dissolved in the water to significant concentrations. All three compounds volatilized within 24 h and, thus, did not remain in the water long enough for microbial degradation to affect their fate. Inclusion of sediment (500 mg/l dry weight) did not retard the disappearance of the fuel components, and rates of disappearance were identical in controls sterilized with $HgCl_2$.

The lack of biodegradation was unexpected because the simple aromatic hydrocarbons are readily biodegradeable under aerobic conditions (1). We measured the numbers of both heterotrophic and hydrocarbonoclastic microorganisms during the incubation period to test whether the fuel was toxic to the resident microflora (Fig. 1). There were no detectable differences between populations in treated and control flasks. The fuel was neither stimulatory, nor toxic, to the microbes. The results indicate that the interactions between the fuel and the microflora were minimal, even in the presence of suspended sediment. Bacteria, when exposed for the first time to a biodegradeable substrate, often require an acclimation period before they degrade the compound at a significant rate. The length of the acclimation period can thus be an important consideration in biodegradation studies (7). In this instance the fuel was not present in the water long enough to cause acclimation of the microorganisms.

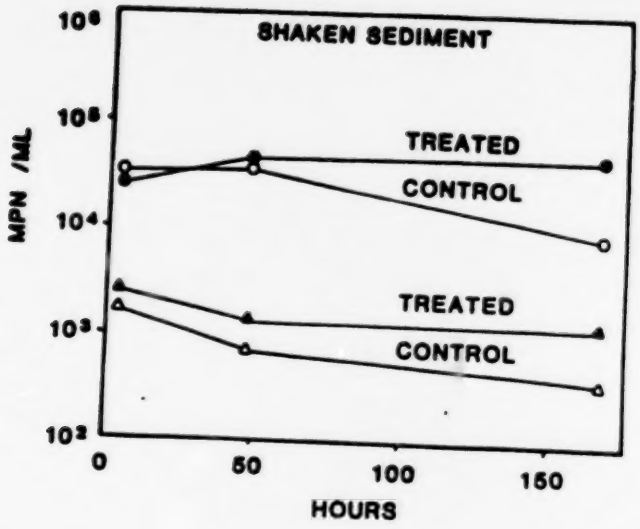


Figure 1. Effects of JP-4 on microbial community size during biodegradation test. Sediment and water were from Range Point saltmarsh. Heterotrophic (upper curves) and hydrocarbonoclastic (lower curves) microorganisms were enumerated as described in Methods.

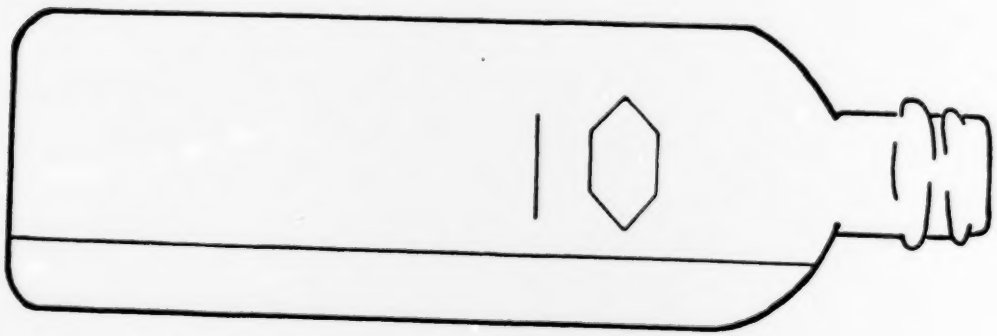


Figure 2. Quiescent test system used in biodegradation studies. Fuel was incubated with 25 ml sediment and water samples in 150 ml milk dilution bottles.

The above experiments suggested that the long-term effects after release of JP-4 in open water would be minimal. The fuel would volatilize rapidly and have little effect on the resident microflora.

Shallow aquatic habitats such as estuaries, rivers, and saltmarshes are characterized by high suspended sediment loads and bed sediment near the surface of the water. Fuels released in such situations can be expected to interact readily with sediment. We used milk dilution bottles (Fig. 2) containing water and sediment to study the fate of fuel in a shallow aquatic habitat. In initial experiments, designed to simulate calm conditions in the field, we gently layered JP-4 on the surface of the water and incubated the bottles with no agitation. The fuel volatilized from the bottles rapidly regardless of the presence of sediment which indicates that it did not partition into the sediment.

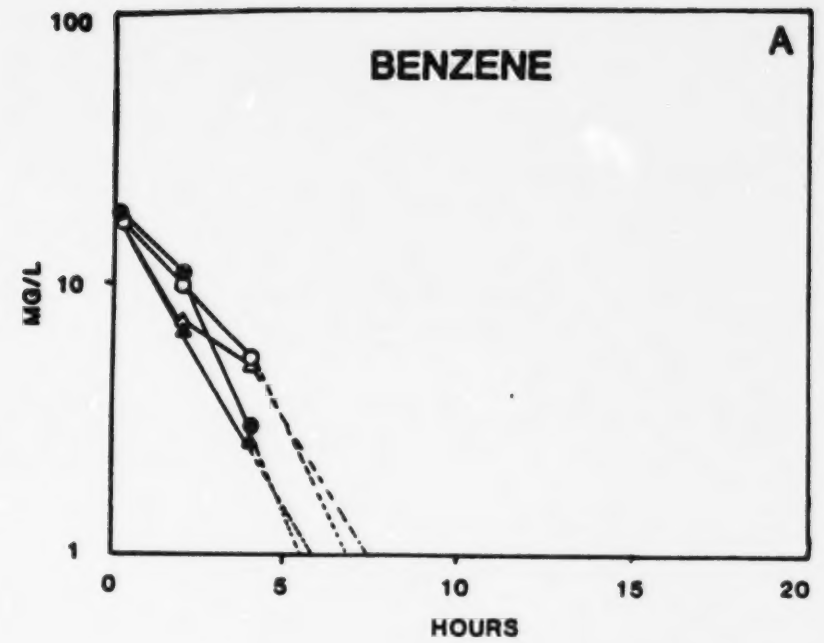
Because jet fuels are transported extensively by barges, the most likely circumstances for accidental release involve shallow turbulent water in rivers and estuaries. Previous fuel spills have persisted and caused environmental problems when released during stormy weather (4). Under such circumstances the fuels become sorbed to suspended sediment which settles and becomes a sink for the fuel. We designed laboratory experiments to study how interactions with sediment affected volatility and biodegradation in water and sediment from the Escambia River. We used the same vessels as for earlier experiments but shook the suspensions vigorously after the addition of the fuel to allow sediment-fuel interactions. Four types of treatment allowed us to assess the relative contributions of the various processes affecting the rate of the fuel. Bottles with river water allowed assessment of biodegradation and volatilization. Sediment was included in some of the bottles so that the effects of sorption and sediment-associated microorganisms could be

evaluated. Controls were sterilized so that abiotic processes could be evaluated in the absence of biodegradation.

Benzene, toluene, and cyclohexane volatilized from all test bottles within 10 h (Fig. 3a). The more substituted benzenes become associated with sediment and were less affected by volatilization. p-Xylene, for example, volatilized quickly from water (Fig. 3b) but much more slowly from bottles containing sediment. In bottles with non-sterile sediment the fuel components disappeared much faster than in sterile controls. Because the opportunities for sorption and volatilization were the same in the two systems we attributed the faster disappearance to biodegradation. The sediment held the fuel components in the system long enough for the microorganisms to acclimate and for biodegradation to become significant. In addition, the sediment-associated microorganisms probably also contributed significantly to the biodegradation of the hydrocarbons. The results were similar for ethylbenzene, 1-methyl,3-ethylbenzene and 1,2,4-trimethylbenzene; the only difference was the length of time required for acclimation of the resident microbial community.

n-Decane (Fig. 3c), 1,4-dimethyl,2-ethyl-benzene, n-undecane (Fig. 3d), naphthalene (Fig. 3e), and n-dodecane were biodegraded in both water and water-sediment mixtures. Because of their lower volatilization rates these compounds remained in water long enough to cause acclimation of the resident microbial communities. Sediment further reduced the volatilization of these hydrocarbons but also seemed to inhibit their biodegradation. There was some biodegradation in bottles containing sediment but not as much as in water alone.

We did not detect biodegradation of substituted naphthalenes or linear alkanes in any of the experimental systems. Tridecane (Fig. 3f) for example,



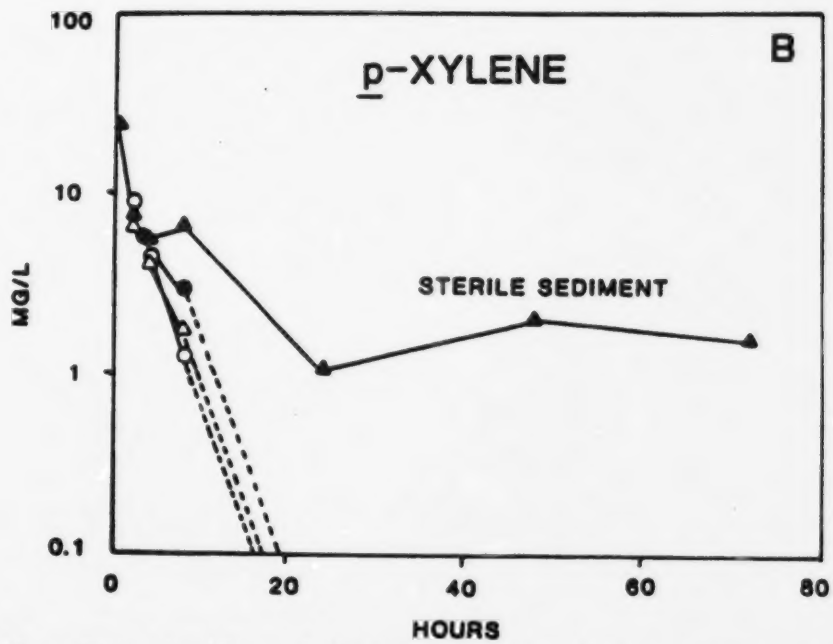
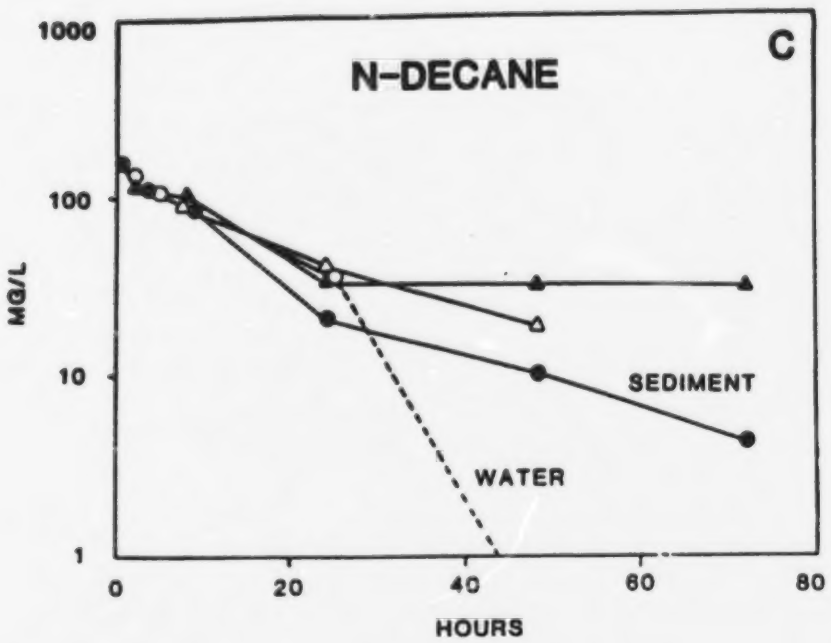


Figure 3. Concentration of fuel components during biodegradation test with Escambia River sediment and water. Fuel was added to test bottles containing: ○, water; ∧, sterile water; ◕, sediment and water; ▲, sterile sediment and water [*(a) Benzene; (b) p-Xylene; (c) N-Decane; (d) Undecane; (e) Napthalene; (f) Tridecane*].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N-DECANE
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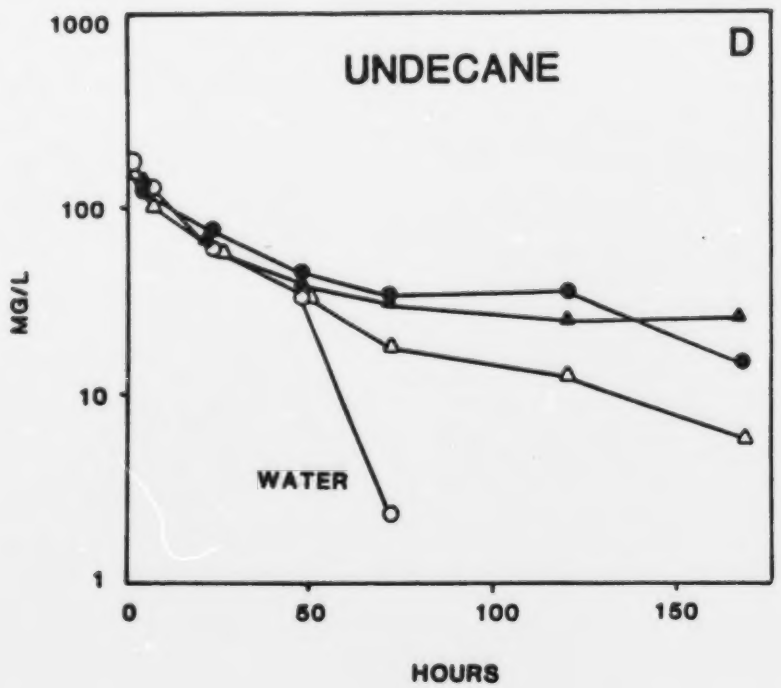
40
60
80
HOURS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10
1
MG/L
WATER
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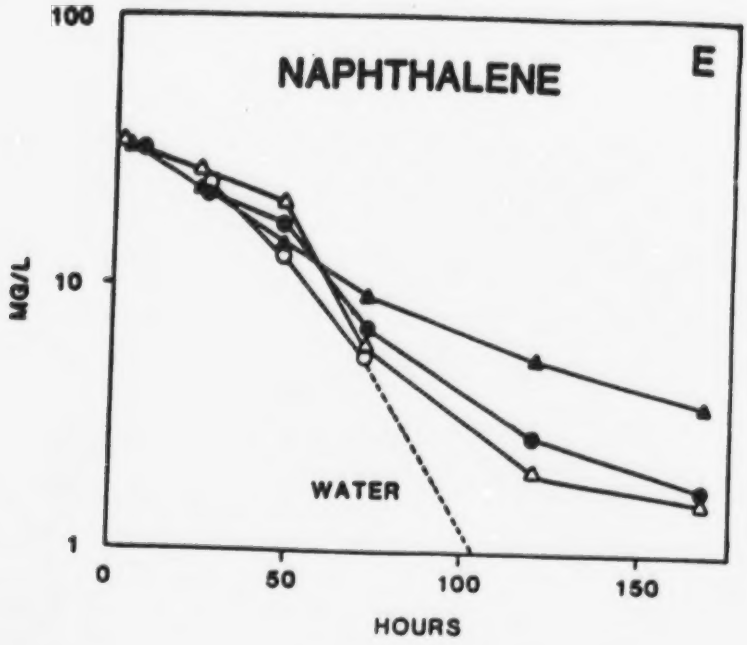
100
150
HOURS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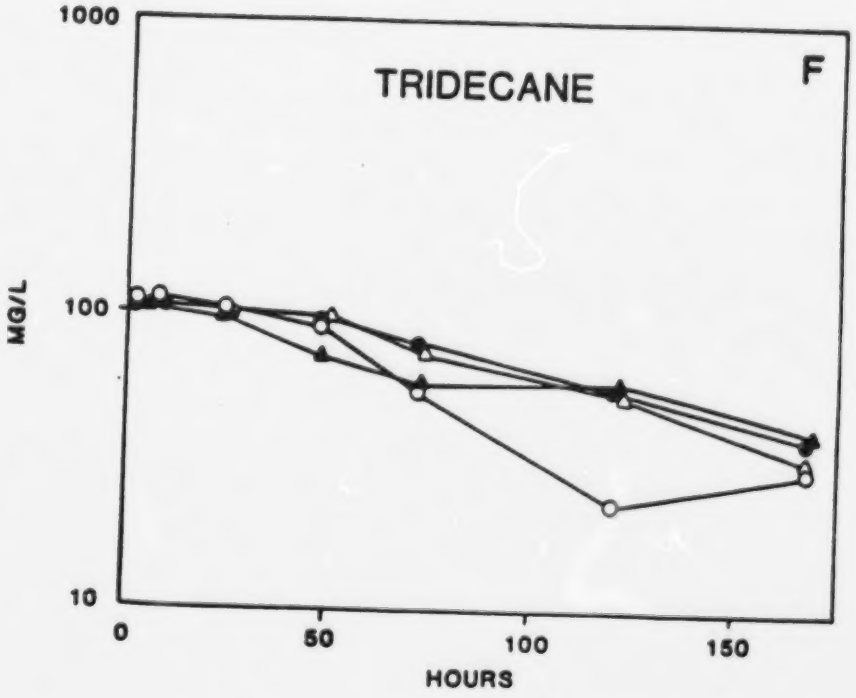
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150
HOURS

slowly volatilized during the incubation period and was not affected by the presence of sediment or active microorganisms.

Table 1 summarizes the fate of the selected JP-4 components in sediment and water from the river. The lightest compounds volatilized rapidly and their fate was not affected by biodegradation or by interactions with sediment. Interactions with sediment held the substituted benzenes in contact with the microflora long enough for biodegradation to become important. The next heavier compounds from n-decane through n-dodecane volatilized more slowly. They stimulated microbial acclimation in the water; acclimation and subsequent biodegradation were inhibited by sediment. The substituted naphthalenes and largest linear alkanes volatilized slowly and their disappearance rates were unaffected by biodegradation or sorption to sediment.

JP-4 affected the microbial community in the quiescent experimental systems more than in shake flasks because the microbes were exposed to higher concentrations of the fuel for longer periods. JP-4 was initially toxic to bacteria in water (Fig. 4a). During subsequent incubation populations of both hydrocarbon degraders and the heterotrophs were stimulated by the fuels which suggests that as they became acclimated they were able to use the fuel components as sources of carbon and energy. The response of the microorgansims was similar in bottles containing sediment (Fig. 4b) except that the initial toxicity was slightly lower.

When we repeated the experiment with sediment and water from the Range Point saltmarsh, the sediment again reduced the volatilization of some of the intermediate compounds (Table 2). There was no significant biodegradation, however, of any of the fuel components in the bottle containing sediment. Results with undecane (Fig. 5) were typical of those obtained with saltmarsh sediment and water. Undecane volatilized slightly faster in bottles without

Table 1. Fate of selected JP-4 components in sediment and water from Escambia River.

Compound	Site of Biodegradation
BENZENE	
CYCLOHEXANE	VOLATILIZED
TOLUENE	
ETHYLBENZENE	
p-XYLENE	SEDIMENT
1-METHYL,3-ETHYLBENZENE	
1,2,4-TRIMETHYLBENZENE	
n-DECANE	WATER AND
1,4-DIMETHYL,2-ETHYLBENZENE	SEDIMENT
n-UNDECANE	
NAPHTHALENE	WATER
n-DODECANE	
2-METHYLNAPHTHALENE	
n-TRIDECANE	VOLATILIZED
n-TETRADECANE	

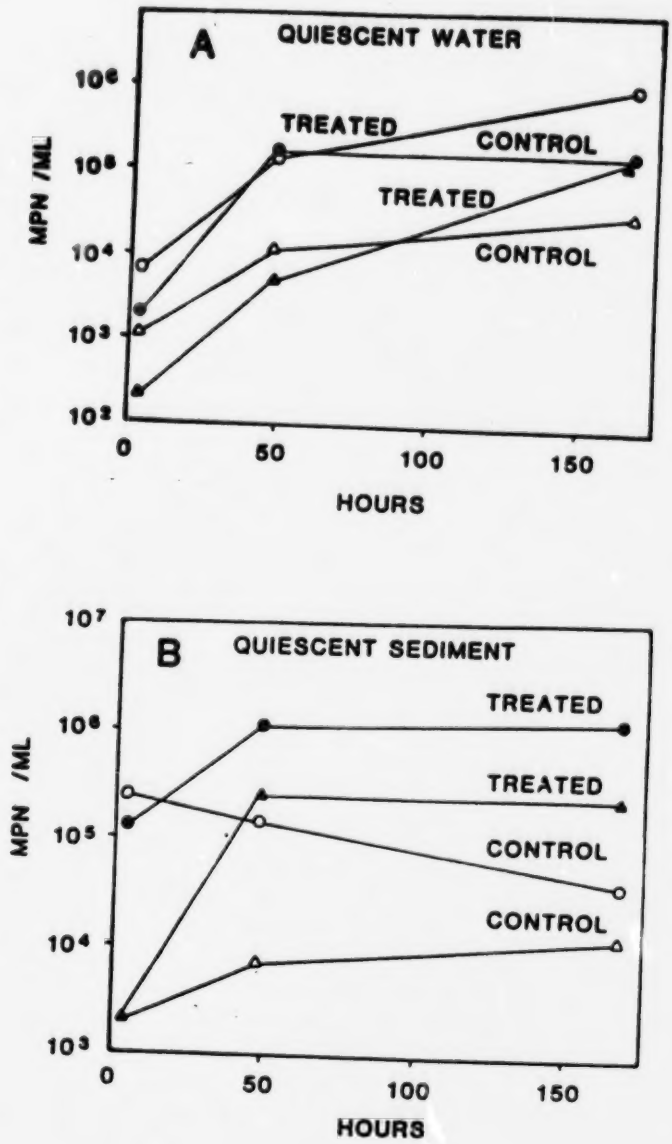

Figure 4. Effect of JP-4 on microbial community size during quiescent biodegradation test. Heterotrophic (circles) and hydrocarbonoclastic (triangles) microorganisms were enumerated as described in Methods.

Table 2. Fate of selected JP-4 components in sediment and water from Range Point saltmarsh.

Compound	Site of Biodegradation
BENZENE	
CYCLOHEXANE	VOLATILIZED
TOLUENE	
ETHYLBENZENE	WATER
p-XYLENE	VOLATILIZED
1-METHYL,3-ETHYLBENZENE	
1,2,4-TRIMETHYLBENZENE	
n-DECANE	WATER
1,4-DIMETHYL,2-ETHYLBENZENE	
n-UNDECANE	
NAPHTHALENE	VOLATILIZED
n-DODECANE	
2-METHYLNAPHTHALENE	WATER
n-TRIDECANE	
n-TETRADECANE	WATER AND SEDIMENT

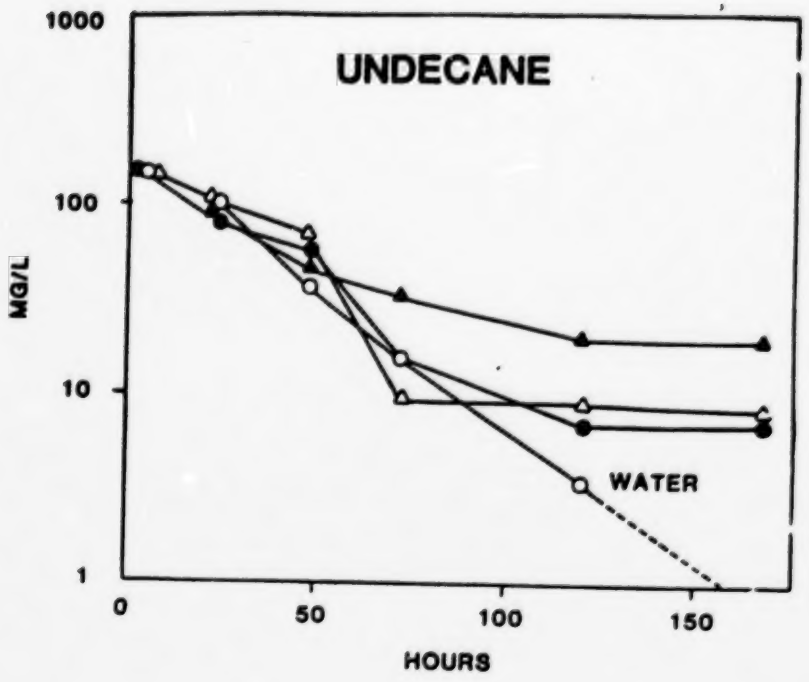

Figure 5. Undecane concentration during quiescent biodegradation test with Range Point sediment and water.

sediment. After several days the microbial community in the water acclimated and the hydrocarbons were biodegraded rapidly. Thus, while interactions between sediment and fuels reduced volatilization, they also inhibited biodegradation.

History of exposure can affect the level of hydrocarbonoclastic bacteria in a microbial community and thus the biodegradation of hydrocarbons in an ecosystem (5, 8). The saltmarsh and river sampling sites had little previous exposure to hydrocarbons. It seemed that the response of a microbial community from a more polluted area would be more predictable and that hydrocarbon biodegradation would be more likely. Therefore, we examined the fate of JP-4 in sediment and water from Bayou Chico, an industrialized area containing several marinas and receiving runoff from industrial sites. Volatilization seemed to be the only process that affected the fate of the fuel components we assayed. Sediment slowed the volatilization of some of the intermediate compounds. There was no evidence of biodegradation of the fuel components during the 7-day incubation period. The lack of biodegradation was unexpected and could not be explained by differences in populations of bacteria or any other obvious parameter. Initial microbial populations were similar in size to those from the river and saltmarsh. Perhaps some undetected contaminant in the water inhibited hydrocarbon degradation or microbial activity in general.

CONCLUSIONS

The effects of abiotic processes such as volatilization, sorption, and partitioning on the fate of petroleum hydrocarbons are predictable. The role of biodegradation and the factors that control biodegradation are poorly understood and should be further studied. We cannot predict how microbial communities will respond to certain hydrocarbons. In situations where communities acclimate to degrade hydrocarbons it is not clear what factors control the length of the acclimation period. Indeed, compounds can be biodegraded readily under one set of conditions and recalcitrant under another, seemingly similar, set of conditions. Therefore, each situation should be considered separately and caution should be exercised in extrapolation of results from one system to another.

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